REMARKS

This paper is being filed in response to the Office Action dated March 25, 2004 that was issued in the above-identified application. Applicants respectfully request reconsideration of the above-identified application in light of the amendments and remarks presented in the instant Amendment.

Claims 42-51, 53, 55-56, 82, and 85-86 were pending. Claim 44 has been cancelled and claims 46-48, 50-51, 53, and 82 have been amended. The amendments are supported by the application as originally filed and, therefore, do not constitute new matter. Upon entry of the instant Amendment, claims 42, 43, 45-51, 53, 55-56, 82, and 85-86 will continue to be pending.

1. Priority Claim to Middlebrook Is Proper

Applicant's claim for priority to U.S. Patent Application No. 08/123,975 filed on September 21, 1993 (hereinafter "the '975 application") has been rejected. The Examiner has alleged that the '975 application was abandoned on June 20, 2001 while the instant application was filed on July 20, 2001. According to the Examiner, the instant application is allegedly not entitled to priority to the '975 application because the two applications were not co-pending.

Applicants traverse this rejection and assert that the instant application is entitled to the benefit of the '975 application. Applicants respectfully invite the Examiner's attention to MPEP § 201.11(III)(C), which discusses priority claims in a series of related applications. The MPEP states that "[i]f applicant wishes that the pending application have the benefit of the filing date of the first filed application, applicant must, besides making reference to the intermediate

application, also make reference to the first application." *See* MPEP § 201.11(III)(C). Therefore, the instant application need not have been co-pending with the '975 application, so long as it claims priority to an intermediate application that was co-pending with the '975 application.

Applicants respectfully invite the Examiner's attention to the Official Filing Receipt issued in connection with the present application and note that this application is a continuation of U.S. Application No. 09/611,419 filed **July 6, 2000** (hereinafter "the '419 application"). Although the '975 application was abandoned on June 20, 2001, it is clear that this date was **after** the date in which the '419 application was filed. Accordingly, the '419 application and the '975 application were co-pending. Next, Applicants respectfully invite the Examiner's attention to the Declaration filed in connection with the '419 application, wherein priority is claimed to the '975 application. Applicants assert, therefore, that under 35 U.S.C. § 120 and MPEP § 201.11(III)(C), the instant application is entitled to claim the benefit of the '975 application, not by virtue of its own co-pendency therewith, but rather on the basis of the co-pendency of the '419 application (of which the present application is a continuation application) and the '975 application. Thus, because no break in the chain of pendency has occurred, the claimed benefit of priority is proper as a matter of right. As such, Applicants respectfully request withdrawal of this rejection, which moots any art-based rejection dated later than September 21, 1992 (see also below).

2. Incorporation By Reference Proper

The Examiner has alleged that Applicant's attempt to incorporate subject matter by reference to Whelan et al. (1992, *Applied and Environmental Microbiology*)(hereinafter "Whelan") and Thompson (1990, European Journal of Biochemistry)(hereinafter "Thompson") is improper. The material sought to be incorporated allegedly is "essential" and, therefore, must be accompanied by a declaration stating that the amendatory material consists of the same material incorporated by reference in the referencing application.

Applicants respectfully traverse this rejection and assert that the amendatory material is accompanied by an appropriate affidavit. Applicant's Amendment filed March 7, 2003 included a Second Substitute Sequence Listing with sequences that corrected minor errors in the previously filed sequences. In addition, Applicant's March 7, 2003 Amendment deleted the sequences presented in the text of the description in favor of simple references to the respective sequence identification numbers. Applicant's Amendment filed December 3, 2003 included a Third Substitute Sequence Listing, which corrected minor errors remaining in the Second Substitute Sequence Listing. Therefore, any subject matter that was introduced to the present application by the Second Substitute Sequence Listing was necessarily removed from the application by Applicant's Amendment filed December 3, 2003.

Applicants referred to the sequences presented in Whelan and Thompson to support the amended sequences presented in the Third Substitute Sequence Listing. In addition, Applicants invited the Examiner's attention to page 44, line 18-20 of the specification, which states "All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually

indicated to be incorporated by reference." Applicants referred to the incorporation by reference merely to buttress Applicant's argument that it was proper to look to Whelan and Thompson to support the amendments to SEQ ID NOS:40, 41, and 42. Thompson discloses the full-length sequence of the serotype A heavy chain and Whelan discloses the full-length sequence of the serotype B heavy chain. However, the carboxy-terminal fragments were recognized as such, by Das Gupta et al. (1990, *Biochemie* **72**:661-664) (serotype A) (hereinafter "Das Gupta") and Schmidt et al. (1985, *Arch. Biochem. Biophys.* 238:544-548) (serotype B) (hereinafter "Schmidt").

Since Thompson and Whelan disclose the full-length sequence of the serotype A heavy chain and B heavy chain, respectively, Applicants did not attempt to literally incorporate the entire sequences of both into the instant application. Rather, as shown in alignments included in both the Amendment filed March 7, 2003 and the Amendment filed December 3, 2003, Applicants were merely calling attention to the sequence of the carboxy-terminal fragment of Thompson and Whelan.

Nevertheless, Applicant's undersigned representative, hereby affirms that the amendatory material in SEQ ID NOS:40-42 of the Third Substitute Sequence Listing, namely the carboxy-terminal portion of the heavy chain of botulinum neurotoxin serotypes A and B, consists of the same material incorporated by reference to the carboxy-terminal portion of the heavy chain of botulinum neurotoxin serotype A as disclosed by Thompson and Das Gupta and serotype B as disclosed by Whelan and Schmidt. Thus, this amendatory material consists of the same material incorporated by reference in the referencing application. Accordingly, Applicants assert that the instant application fully complies with MPEP § 608.01(p), page 600-80, subparagraph 2 and thereby obviates this objection.

3. Objection to Disclosure Obviated

Applicant's Amendment filed on March 7, 2003 is objected under 35 U.S.C. § 132 as allegedly introducing new matter to the instant disclosure. This objection is based on the Examiner's allegation that a priority claim to the '975 application is improper and that incorporation of material from Thompson and Whelan by reference was improper.

Applicants traverse this objection and assert that Applicant's Amendment filed March 7, 2004 did not introduce new matter to the instant application. As set forth above, Applicant's claim for priority to the '975 application via the '419 application is proper. Therefore, Applicants respectfully request withdrawal of this objection and acknowledgement of the validity of the claim for priority to the '975 application.

With respect to the incorporation of material from Thompson and Whelan, Applicants respectfully assert that this objection is misdirected in that the amendatory material in Applicant's Amendment filed March 7, 2003 to which the Examiner has referred was rescinded by Applicant's submission the Third Substitute Sequence Listing included with Applicant's Amendment dated December 3, 2003. Thus, any objection to material in Applicant's Second Substitute Sequence Listing is moot. Applicants have herewith submitted a proper affirmation for incorporation of material from Thompson and Whelan by reference. Therefore, Applicants believe that nothing in the Third Substitute Sequence Listing constitutes new matter and, accordingly, respectfully request withdrawal of this objection.

4. Objection Moot In View of Inclusion of Table 1 with Original Application Papers

The Examiner has objected to the instant disclosure as allegedly failing to include the Table 1 referenced at page 14, lines 12-28. Applicants respectfully traverse this objection and assert that a copy of Table 1 was submitted along with the application papers originally filed in this application. Applicants enclose herewith a copy of Table 1 as originally filed for the Examiner's inspection. Applicants also enclose herewith a copy of Applicant's Return Postcard bearing a PTO Mailroom stamp. As the postcard reflects, the full-page Table 1 was bundled and numbered with the drawings. Applicants also respectfully invite the Examiner's attention to the '419 application, the parent of the instant application, and note that a copy of Table 1 is also found therein.

Upon reviewing the appearance of the typeface of Table 1, Applicants have chosen to replace Table 1 herein to improve the clarity of the characters and eliminate some text. This amendment is fully supported by the application as filed and, therefore, does not introduce any new matter. Applicants respectfully request withdrawal of this objection as being moot in view of the fact that Table 1 appeared in the application as originally filed.

5. Claims Are Clear and Definite

Claims 42-51, 53, 55-56, 82, and 85-86 have been rejected under 35 U.S.C. § 112, second paragraph as allegedly indefinite. Claims 46 and 47 have been rejected as allegedly indefinite for lacking antecedent basis for the phrase "expression control sequence." Claim 48 is allegedly indefinite for being an incomplete method. Claims 50 and 51 are allegedly unclear for

lacking proper antecedent basis for the term "organism." Claim 53 is allegedly unclear for lacking proper antecedent basis for the term "recovered polypeptide."

Applicants respectfully traverse these rejections and assert that these claims, as amended herein, are clear and definite and, therefore, respectfully request withdrawal of these rejections.

Claims 49 and 53 are allegedly unclear for reciting the term "insoluble polypeptide." The Examiner has alleged that the claims do not recite the structural features that render the polypeptide insoluble. In addition, the Examiner has alleged that the specification does not clearly indicate that the polypeptide itself is insoluble, merely that it is recovered in an insoluble fraction. These claims have also been alleged to be unclear for failing to indicate the source of the recited epitope.

Applicants respectfully traverse these rejections and assert that claims 49 and 53 are clear and definite. Regarding the insolubility of botulinum neurotoxins, while Applicants agree that the disclosure identifies the fraction in which the polypeptide is found as "the insoluble fraction," Applicants assert that the specification identifies the polypeptide itself as insoluble at, for example, page 21, lines 9-10, which states "This suggests an alternative process whereby insoluble rBoNT product produced in yeast may be resolubilized and purified to homogeneity." Applicants also assert that providing the amino acid sequence, *i.e.* the primary structure of the polypeptide, alone constitutes a sufficient description of the structural features that may give rise to insolubility. Accordingly, the claim language is clear and definite.

With respect to the source of the epitope, Applicants respectfully invite the Examiner's attention to the precise language of the claims, which both state "at least one insoluble polypeptide comprising the **amino acid sequence** of SEQ ID NO:8, said **amino acid**

sequence having at least one immunogenic epitope" (emphasis added). This language clearly indicates that it is the "amino acid sequence" that has an epitope and that this "amino acid sequence" is the amino acid sequence of SEQ ID NO:8. Thus, despite any allegation to the contrary, the source of the epitope is clearly indicated to be within SEQ ID NO:8.

Claims 42-51, 53, 55-56, 82, and 85-86 have been rejected as allegedly indefinite for reciting the phrase "encoding a polypeptide having the amino acid sequence of SEQ ID NO:8, said amino acid sequence comprising at least one immunogenic epitope." The Examiner has alleged that the specification has defined the invention to include or comprise peptide fragments having protective epitopes at pages 15-16 and 24 of the specification. According to the Examiner, reciting the structure of the polypeptide to be SEQ ID NO:8 and reciting "at least one immunogenic epitope" is allegedly confusing and constitutes an internal inconsistency. The inconsistency arises from the fact that SEQ ID NO:8, as the full-length carboxy-terminal heavy chain sequence, allegedly comprises a plurality of epitopes, yet the recital of a single epitope changes the invention to fragments of SEQ ID NO:8.

Applicants traverse this rejection and assert that these claims are clear and definite. The present invention relates to recombinant botulinum neurotoxin. According to some embodiments of the invention, the recombinant polypeptide may be isolated from cell lysate in either a soluble fraction or an insoluble fraction. *See e.g.* page 20, line 19 *et seq*. In the latter case, the recombinant protein may be reconstituted by adding urea to the insoluble protein followed by dialyzing the urea. Applicants submit that when performed properly, the resulting polypeptide will have its native structure and, accordingly, all of the native epitopes. However, it is well within the contemplation of the instant invention to inadvertently or deliberately modify the reconstitution protocol to yield a partially reconstituted polypeptide with less than the full

complement of epitopes of the properly folded molecule. Thus, it is entirely possible that a polypeptide having the entire amino acid sequence of SEQ ID NO:8 could have as few as one immunogenic epitope.

Moreover, Applicants respectfully disagree with the allegation that simply reciting the phrase "at least one immunogenic epitope" redefines the claim to be directed to peptide fragments. On the contrary, Applicant's assert that this constitutes a functional limitation that is perfectly compatible with the recited structural definition. *See* MPEP § 2173.05(g) ("There is nothing inherently wrong with defining some part of an invention in functional terms. Functional language does not, in and of itself, render a claim improper"). It also indirectly constitutes a structural limitation. The amino acid sequence defines the primary structure of a polypeptide. However, polypeptides also have secondary, tertiary, and quaternary structure. It is often the structure as a whole that impacts or even determines whether or not the polypeptide has one or more epitopes. Thus, reciting "at least one immunogenic epitope" communicates that polypeptides of the invention, in addition to having the sequence of SEQ ID NO:8, also have sufficient higher order structure to give rise to an epitope. For the foregoing reasons, Applicants respectfully request withdrawal of the instant rejection.

Claims 85-86 are allegedly indefinite for reciting the phrases "wherein said polypeptide is at least 0.75% (w/w) of the total cellular protein" (claim 85 and "wherein said polypeptide is at least 20% (w/w) of the total cellular protein" without accounting for the expression of the encoding nucleic acid. The Examiner has also alleged that the phrase "total cellular protein" lacks antecedent basis in any of the claims on which claims 85-86 depend.

Applicants traverse this rejection and assert that claims 85-86 are clear and definite. Applicants have amended claim 82 to recite "wherein said nucleic acid is expressed"

thereby accounting for the formation of the polypeptide. With respect to the alleged lack of antecedent basis for "total cellular protein," according to the MPEP, failure to provide antecedent basis for terms does not always render the claim indefinite. *See* MPEP § 2173.05(e). More specifically, inherent components of recited elements are deemed to have antecedent basis simply by virtue of the presence of the components. *See Id.* According to the MPEP, for example, "the outer surface of said sphere" would not require an explicit recital that the sphere had an outer surface. *See Id.* Like the presence of an outer surface on a sphere, one of ordinary skill in the art would recognize that living cells contain proteins without express recital of such. Accordingly, Applicants respectfully request withdrawal of this rejection.

6. Halpern Does Not Teach SEQ ID NO:8

Claims 42-47, 55-56, and 82-86 have been rejected under 35 U.S.C. §102(a) as allegedly unpatentable over Halpern et al., 1993, *J. Biol. Chem.* 268(15):11188-11192 (hereinafter "Halpern"). The Examiner has alleged that Halpern discloses a nucleic acid that has a nucleotide sequence encoding the carboxy-terminal portion of a botulinum neurotoxin, wherein the nucleic acid encodes an amino acid sequence that is conserved across Clostridial neurotoxins including serotype B. The Examiner has also alleged that Halpern discloses a nucleic acid encoding the amino acid sequence Cys-Cys-Asp-Glu-Gly-Trp-Thr. In addition, Halpern allegedly discloses antibody and immunogenic composition preparation and nucleic acid expression with a T7 RNA polymerase promoter. It has been further alleged that Halpern discloses expression of the nucleic acids of Halpern in recombinant mammalian host cells as well as recovery of the expressed protein.

Applicants traverse this rejection and assert that the claims, as amended herein, are not anticipated by Halpern. Applicants note that this rejection was previously asserted in the Office Action dated November 7, 2002 and withdrawn by the Office Action dated June 3, 2003. This rejection has been reasserted herewith without substantial modification and without explanation for its reassertion. *See e.g.* MPEP § 707.07(d) ("the ground of rejection [should be] fully and clearly stated"). Therefore, Applicants respectfully assert that the rejection based on Halpern has not sufficiently apprised Applicants of the nature of the rejection or afforded Applicants an opportunity to reply. *See e.g.* MPEP § 706.02(j); MPEP § 707.07(d). Therefore, Applicants respectfully request clarification of this rejection.

In order to ensure that this response is not deemed incomplete under 37 C.F.R. § 1.111(b), and without prejudice against the foregoing request for clarification of the rejection based on Halpern, Applicants reiterate the grounds for traversing this rejection raised in the Amendment filed March 7, 2003.

Claims 42 and 53 each recite a "nucleic acid encoding a polypeptide having the amino acid sequence of SEQ ID NO:8, said amino acid sequence comprising at least one immunogenic epitope." Applicants respectfully invite the Examiner's attention to Alignment 1 attached hereto which shows that the sequence of Halpern is not the same as the polypeptide sequence of SEQ ID NO:8 of the present specification. Therefore, since Halpern fails to teach SEQ ID NO:8 of the present specification, Halpern fails anticipate each and every element of claims 42 and 53. Consequently, Applicants respectfully request withdrawal of this rejection.

7. Smith Does Not Teach SEQ ID NO:8

Claims 48, and 51 have been rejected under 35 U.S.C. §102(a) as allegedly unpatentable over Smith, 1998, *Toxicon* **36**(11):1539-1548 (hereinafter "Smith 1998"). The Examiner has alleged that Smith 1998 discloses a nucleic acid encoding a Clostridium botulinum type B heavy chain capable of being expressed in *Pichia pastoris*. The Examiner has further alleged that Smith 1998 discloses a method of producing an immunogenic composition comprising culturing a recombinant *Pichia pastoris* cell and recovering the expressed heavy chain polypeptide.

Applicants traverse this rejection and assert that Smith 1998 does not anticipate the claims, as amended herein. Applicants note that this rejection was also previously asserted in the Office Action dated November 7, 2002 and withdrawn by the Office Action dated June 3, 2003. This rejection has been reasserted herewith very little modification and no substantial explanation for its reassertion. *See e.g.* MPEP § 707.07(d) ("the ground of rejection [should be] fully and clearly stated"). Therefore, Applicants respectfully assert that the rejection as presented has not sufficiently apprised Applicants of the nature of the rejection or afforded Applicants an opportunity to reply. *See e.g.* MPEP § 706.02(j); MPEP § 707.07(d). Applicants respectfully request clarification of this rejection.

Again, without prejudice against the foregoing request, Applicants reiterate the grounds for traversing this rejection that were raised in the Amendment filed on March 7, 2003.

Claim 48 recites "transfecting a cell with a nucleic acid having a nucleotide sequence encoding a polypeptide having the amino acid sequence of SEQ ID NO:8, said amino acid sequence comprising at least one immunogenic epitope". Smith 1998 does not teach the

amino acid sequence of SEQ ID NO:8. Since Smith 1998 does not teach each and every element of the claimed invention, Applicants respectfully request withdrawal of this rejection.

In reasserting this rejection, the Examiner has parenthetically alleged that Applicants are not entitled to a priority claim to 1993 with respect to *Pichia pastoris*. As discussed above, Applicants assert that the present application is properly entitled to the benefit of the earliest claimed priority date. As such, Smith 1998 is not properly considered to be prior art with respect to the present application.

8. Whelan Does Not Teach SEQ ID NO:8

Claims 42-50, 55 and 82 have been rejected under 35 U.S.C. §102(b) as allegedly unpatentable over Whelan et al., April 26, 1993, Accession M81186 GI:144743 (hereinafter "Whalen"). The Examiner has alleged that this rejection is made

> ... for reason of record in light of all of the claims reciting the phrase "said amino acid sequence comprising at least one epitope, thus claiming an isolated or purified nucleic acid that encodes an immunogenic epitope of the Hc domain of serotype B botulinum neurotoxin (see Whalen et al, entire reference, especially Figure 3, pages 2350-2351; page 2352, consensus sequence col. 2) showing conserved sequences that include at least one epitope across species of botulinum neurotoxin to include botulinum serotype B neurotoxin. [*sic*, entire quote] Outstanding Office Action dated March 25, 2004, paragraph 28, pages 11-12.

The Examiner has also alleged that the term "about" in the phrase "less than about 70%" in claim 55 would include values of 74.6% since this is within 10% variation. Whalen allegedly discloses sequences with an A+T composition of 74.6%.

Applicants traverse this rejection and assert that the claims, as amended herein, are not anticipated by Whalen. Applicants respectfully assert that the passage of the Office Action quoted above does not sufficiently apprise Applicants of the nature of the rejection or

afford Applicants an opportunity to reply. *See e.g.* MPEP § 706.02(j); MPEP § 707.07(d). Therefore, Applicants respectfully request clarification of this rejection. Without prejudice against the foregoing request, Applicants reiterate the grounds for traversing this rejection raised in the Amendment filed March 7, 2003 in order to more fully ensure that instant response is not deemed incomplete under 37 C.F.R. § 1.111(b).

Claims 42 and 53 each recite a "a nucleotide sequence encoding a polypeptide having the amino acid sequence of SEQ ID NO:8, said amino acid sequence comprising at least one immunogenic epitope". Applicants respectfully invite the Examiner's attention to Alignments 2 and 3 attached hereto, which show that the sequence of Accession No. M81186 is not the same as the sequences of SEQ ID NOS:7 and 8, respectively, of the present invention. Therefore, since Whalen fails to teach the instant SEQ ID NO:8, it fails to anticipate claims 42 and 53. Applicants further contend that the nucleic acid of Whalen, with total AT content of 74.56% and an AT content over the aligned region of 76.45%, would be poorly, if at all, expressible in yeast, gram negative bacteria and mammalian cell lines. *See e.g.* page 14, lines 20-22. Consequently, Applicants respectfully request withdrawal of this rejection.

Conclusion

Applicants believe that the application is in condition for allowance and respectfully request prompt, favorable action. However, should the Examiner disagree, Applicants respectfully submit that the lack of clarity in some of the outstanding rejections militates issuance of another **non-final** office action.

Applicants enclose herewith the fee required under 37 C.F.R. §1.17(a)(2). Although Applicants do not believe that any additional fees are required with this paper, the

Commissioner is hereby authorized to charge any fees occasioned by this submission not otherwise enclosed herewith to Deposit Account No. 02-4377. Please credit any overpayment of fees associated with this filing to the above-identified deposit account. A duplicate of this page is enclosed.

Respectfully submitted,

BAKER BOTTS, L.L.P.

August 25, 2004

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Enclosures

IN THE DRAWINGS

Please **delete** the first sheet of drawings marked as "Table 1."

From : Pichia AOX1 gene
REFORMAT of: aox1struc.dat check: 7028
from: 1 to: 1992 27-MAY-1987 11:40

From : Pichia AOX2 gene
REFORMAT of: aox2strpk.dat check: 9641
from: 1 to: 1992 27-MAY-1987 11:41

From : Pichia DAS1 gene
REFORMAT of: das1struc.dat check: 3191
from: 1 to: 2124 22-APR-1987 15:08

From : Pichia DAS2 gene
REFORMAT of: das2struc.dat check: 5478
from: 1 to: 2124 15-JUN-1987 14:33

From : Pichia GAP gene
REFORMAT of: pgapstruc.dat check: 9059
from: 1 to: 1002 15-JUN-1987 14:38

AmAcid	Codon	Number	Fraction
Gly	GGG	0.00	0.00
Gly	GGA	59.00	0.22
Gly	GGT	197.00	0.74
Gly	GGC	9.00	0.03
Glu	GAG	112.00	0.58
Glu	GAA	80.00	0.42
Asp	GAT	56.00	0.32
Asp	GAC	118.00	0.68
Val	GTG	10.00	0.05
Val	GTA	8.00	0.04
Val	GTT	107.00	0.50
Val	GTC	87.00	0.41
Ala	GCG	1.00	0.00
Ala	GCA	25.00	0.10
Ala	GCT	147.00	0.60
Ala	GCC	71.00	0.29
Arg	AGG	2.00	0.01
Arg	AGA	111.00	0.79
Ser	AGT	8.00	0.04
Ser	AGC	3.00	0.02
Lys	AAG	145.00	0.79
Lys	AAA	38.00	0.21
Asn	AAT	18.00	0.13
Asn	AAC	119.00	0.87
Met	ATG	90.00	1.00
Ile	ATA	0.00	0.00
Ile	ATT	93.00	0.56
Ile	ATC	72.00	0.44
Thr	ACG	5.00	0.03
Thr	ACA	8.00	0.05
Thr	ACT	86.00	0.50
Thr	ACC	74.00	0.43
Trp	TGG	39.00	1.00
End	TGA	0.00	0.00
Cys	TGT	35.00	0.83
Cys	TGC	7.00	0.17
End	TAG	1.00	0.20
End	TAA	4.00	0.80
Tyr	TAT	18.00	0.12
Tyr	TAC	128.00	0.88
Leu	TTG	120.00	0.52
Leu	TTA	21.00	0.09
Phe	TTT	24.00	0.19
Phe	TTC	104.00	0.81
Ser	TCG	8.00	0.03
Ser	TCA	14.00	0.07
Ser	TCT	88.00	0.47
Ser	TCC	71.00	0.37
Arg	CGG	2.00	0.01
Arg	CGA	0.00	0.00
Arg	CGT	26.00	0.18
Arg	CGC	0.00	0.00
Gln	CAG	31.00	0.34
Gln	CAA	59.00	0.66
His	CAT	11.00	0.13
His	CAC	77.00	0.88
Leu	CTG	35.00	0.15
Leu	CTA	7.00	0.03
Leu	CTT	43.00	0.18
Leu	CTC	7.00	0.03
Pro	CCG	0.00	0.00
Pro	CCA	97.00	0.57
Pro	CCT	68.00	0.39
Pro	CCC	7.00	0.04

Table 1

PICHIA CODON USAGE TABLE

33626-A

BAKER BOTTS L.L.P.

01 AUG -9 AM 10:21

TO RKB HHL BFB

File Number: A33626-A

The stamp of the Patent Office Mail Room hereon acknowledges receipt on the date indicated by such stamp of patent application papers comprising 74 page(s) of specification, 7 page(s) of claims, 23 sheet(s) of drawings, a check in the amount of $$2,174, 13 pages of Preliminary Amendment, 53 pages of Sequence Listing, 1 diskette of Sequence Listing and a Declaration by applicant(s) Smith et al.for an invention entitled RECOMBINANT VACCINE AGAINST BOTULINUM NEUROTOXIN

Express Label No.: EK 839 854 ~~832~~ 877 US
Mailing Date: July 20, 2001
Comments:

JC997 U.S. PTO
09/910186
07/20/01

Docketed

For 10/30/2001 By

RS

Alignment 1

Smith SEQ ID NO:8 (hereinafter "Smith8_186) was aligned with the amino acid sequence of Halpern (as shown at p. 11189, col. 1, paragraph 4, line 2; hereinafter "Halpern"). Identical amino acids are marked by an asterisk highlighted in yellow.

```
Smith8_186      MANKYNSEILNNIILNLRYKDNNLIDLSGYGAKVEVYDGVELNDKNQFKLTSSANSKIRVTQNQNIIFNSVFLDFSVSFWIRIPKYKNDGIQNYIHNEYTIINCMKNNSGWKISIRGNRI 120
Halpern         ------------------------------------------------------------------------------------------------------------------------

Smith8_186      IWTLIDINGKTKSVFFEYNIREDISEYINRWFFVTITNNLNNAKIYINGKLESNTDIKDIREVIANGEIIFKLDGDIDRTQFIWMKYFSIFNTELSQSNIEERYKIQSYSEYLKDFWGNP 240
Halpern         ------------------------------------------------------------------------------------------------------------------------

Smith8_186      LMYNKEYYMFNAGNKNSYIKLKKDSPVGEILTRSKYNQNSKYINYRDLYIGEKFIIRRKSNSQSINDDIVRKEDYIYLDFFNLNQEWRVYTYKYFKKEEEKLFLAPISDSDELYNTIQIK 360
Halpern         ------------------------------------------------------------------------------------------------------------------------

Smith8_186      EYDEQPTYSCQLLFKKDEESTDEIGLIGIHRFYESGIVFEEYKDYFCISKWYLKEVKRKPYNLKLGCNWQFIPKDEGWTE 440
Halpern         ---------C----------DKI-------------------------------------LGCDWYFVPTDEGWT- 19
```

Alignment 2

Smith SEQ ID NO:7 (hereinafter "Smith7_186) was aligned with the nucleotide sequence of Whalen (Accession M81186; hereinafter "M81186"). A preliminary alignment (not shown) with the full-length nucleotide sequence of Whalen M81186 revealed very little identity over approximately the first 2580 nucleotides. Therefore, this alignment is limited to only nucleotides 2581-4041. Selection of this region was haphazard with consideration given merely to providing some overlap on either end of the region of highest similarity. Identical nucleotides are marked by an asterisk.

```
Smith7_186      --------------------GAATTCACGATGGCCAACAAATACAATTCCGAAATCCTGAACAATATCATCCTGAACCTGCGTTACAAAGACAACAATCTGATCGATCTGTCTGGTTACG 100
M81186          TATATACCAATGATACAATACTAATAGAAATGTTTAATAAATATAATAGCGAAATTTTAAATAATATTATCTTAAATTTAAGATATAAGGATAATAATTTAATAGATTTATCAGGATATG 2700

Smith7_186      GTGCTAAAGTTGAAGTATACGACGGTGTTGAACTGAATGACAAGAACCAGTTCAAACTGACCTCTTCCGCTAACTCTAAGATCCGTGTTACTCAGAATCAGAACATCATCTTCAACTCCG 220
M81186          GGGCAAAGGTAGAGGTATATGATGGAGTCGAGCTTAATGATAAAAATCAATTTAAATTAACTAGTTCAGCAAATAGTAAGATTAGAGTGACTCAAAATCAGAATATCATATTTAATAGTG 2820

Smith7_186      TATTCCTGGACTTCTCTGTTTCCTTCTGGATCCGTATCCCGAAATACAAGAACGACGGTATCCAGAATTACATCCACAATGAATACACCATCATCAACTGCATGAAGAATAACTCTGGTT 340
M81186          TGTTCCTTGATTTTAGCGTTAGCTTTTGGATAAGAATACCTAAATATAAGAATGATGGTATACAAAATTATATTCATAATGAATATACAATAATTAATTGTATGAAAAATAATTCGGGCT 2940

Smith7_186      GGAAGATCTCCATCCGCGGTAACCGTATCATCTGGACTCTGATCGATATCAACGGTAAGACCAAATCTGTATTCTTCGAATACAACATCCGTGAAGACATCTCTGAATACATCAATCGCT 460
M81186          GGAAAATATCTATTAGGGGTAATAGGATAATATGGACTTTAATTGATATAAATGGAAAAACCAAATCGGTATTTTTTGAATATAACATAAGAGAAGATATATCAGAGTATATAAATAGAT 3060

Smith7_186      GGTTCTTCGTTACCATCACCAATAACCTGAACAATGCTAAAATCTACATCAACGGTAAACTGGAATCTAATACCGACATCAAAGACATCCGTGAAGTTATCGCTAACGGTGAAATCATCT 580
M81186          GGTTTTTTGTAACTATTACTAATAATTTGAATAACGCTAAAATTTATATTAATGGTAAGCTAGAATCAAATACAGATATTAAAGATATAAGAGAAGTTATTGCTAATGGTGAAATAATAT 3180

Smith7_186      TCAAACTGGACGGTGACATCGATCGTACCCAGTTCATCTGGATGAAATACTTCTCCATCTTCAACACCGAACTGTCTCAGTCCAATATCGAAGAACGGTACAAGATCCAGTCTTACTCCG 700
M81186          TTAAATTAGATGGTGATATAGATAGAACACAATTTATTTGGATGAAATATTTCAGTATTTTTAATACGGAATTAAGTCAATCAAATATTGAAGAAAGATATAAAATTCAATCATATAGCG 3300

Smith7_186      AATACCTGAAAGACTTCTGGGGTAATCCGCTGATGTACAACAAAGAATACTATATGTTCAATGCTGGTAACAAGAACTCTTACATCAAACTGAAGAAAGACTCTCCGGTTGGTGAAATCC 820
M81186          AATATTTAAAAGATTTTTGGGGAAATCCTTTAATGTACAATAAAGAATATTATATGTTTAATGCGGGGAATAAAAATTCATATATTAAACTAAAGAAAGATTCACCTGTAGGTGAAATTT 3420

Smith7_186      TGACTCGTTCCAAATACAACCAGAACTCTAAATACATCAACTACCGCGACCTGTACATCGGTGAAAAGTTCATCATCCGTCGCAAATCTAACTCTCAGTCCATCAATGATGACATCGTAC 940
M81186          TAACACGTAGCAAATATAATCAAAATTCTAAATATATAAATTATAGAGATTTATATATTGGAGAAAAATTTATTATAAGAAGAAAGTCAAATTCTCAATCTATAAATGATGATATAGTTA 3540

Smith7_186      GTAAAGAAGACTACATCTACCTGGACTTCTTCAACCTGAATCAGGAATGGCGTGTATACACCTACAAGTACTTCAAGAAAGAAGAAGAAAAGCTTTTCCTGGCTCCGATCTCTGATTCCG 1060
M81186          GAAAAGAAGATTATATATATCTAGATTTTTTTAATTTAAATCAAGAGTGGAGAGTATATACCTATAAATATTTTAAGAAAGAGGAAGAAAAATTGTTTTTAGCTCCTATAAGTGATTCTG 3660

Smith7_186      ACGAACTCTACAACACCATCCAGATCAAAGAATACGACGAACAGCCGACCTACTCTTGCCAGCTGCTGTTCAAGAAAGATGAAGAATCTACTGACGAAATCGGTCTGATCGGTATCCACC 1180
M81186          ATGAGTTTTACAATACTATACAAATAAAAGAATATGATGAACAGCCAACATATAGTTGTCAGTTGCTTTTTAAAAAAGATGAAGAAAGTACTGATGAGATAGGATTGATTGGTATTCATC 3780

Smith7_186      GTTTCTACGAATCTGGTATCGTATTCGAAGAATACAAAGACTACTTCTGCATCTCCAAATGGTACCTGAAGGAAGTTAAACGCAAACCGTACAACCTGAAACTGGGTTGCAATTGGCAGT 1300
M81186          GTTTCTACGAATCTGGAATTGTATTTGAAGAGTATAAAGATTATTTTTGTATAAGTAAATGGTACTTAAAAGAGGTAAAAAGGAAACCATATAATTTAAAATTGGGATGTAATTGGCAGT 3900

Smith7_186      TCATCCCGAAAGACGAAGGTTGGACCGAATAGTAAGAATTC----------------------------------------------------------------------------
M81186          TTATTCCTAAAGATGAAGGGTGGACTGAATAATATAACTATATGCTCAGCAAACCTATTTTATATAAGAAAAGTTTAAGTTTATAAAATCTTAAGTTTAAGGATGTAGCTAAATTTTGAA 4020

Smith7_186      ----------
M81186          TATTAGATAAACTACATGTTT 1461
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Alignment 3

Smith SEQ ID NO:8 (hereinafter "Smith8_186) was aligned with the amino acid sequence of Whalen (Accession M81186; hereinafter "M81186"). A preliminary alignment (not shown) with the full-length nucleotide sequence of Whalen M81186 revealed very little identity over approximately the first 840 amino acids. Therefore, this alignment is limited to only amino acids 841-1291. Selection of this region was haphazard with consideration given merely to providing some overlap on either end of the region of highest similarity. Identical amino acids are marked by an asterisk.

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Smith8_186      ----------MANKYNSEILNNIILNLRYKDNNLIDLSGYGAKVEVYDGVELNDKNQFKLTSSANSKIRVTQNQNIIFNSVFLDFSVSFWIRIPKYKNDGIQNYIHNEYTIINCMKNNS 109
M81186          SIYTNDTILIEMFNKYNSEILNNIILNLRYKDNNLIDLSGYGAKVEVYDGVELNDKNQFKLTSSANSKIRVTQNQNIIFNSVFLDFSVSFWIRIPKYKNDGIQNYIHNEYTIINCMKNNS 960
                          * ********************************************************************************************************

Smith8_186      GWKISIRGNRIIWTLIDINGKTKSVFFEYNIREDISEYINRWFFVTITNNLNNAKIYINGKLESNTDIKDIREVIANGEIIFKLDGDIDRTQFIWMKYFSIFNTELSQSNIEERYKIQSY 229
M81186          GWKISIRGNRIIWTLIDINGKTKSVFFEYNIREDISEYINRWFFVTITNNLNNAKIYINGKLESNTDIKDIREVIANGEIIFKLDGDIDRTQFIWMKYFSIFNTELSQSNIEERYKIQSY 1080
                ************************************************************************************************************************

Smith8_186      SEYLKDFWGNPLMYNKEYYMFNAGNKNSYIKLKKDSPVGEILTRSKYNQNSKYINYRDLYIGEKFIIRRKSNSQSINDDIVRKEDYIYLDFFNLNQEWRVYTYKYFKKEEEKLFLAPISD 349
M81186          SEYLKDFWGNPLMYNKEYYMFNAGNKNSYIKLKKDSPVGEILTRSKYNQNSKYINYRDLYIGEKFIIRRKSNSQSINDDIVRKEDYIYLDFFNLNQEWRVYTYKYFKKEEEKLFLAPISD 1200
                ************************************************************************************************************************

Smith8_186      SDELYNTIQIKEYDEQPTYSCQLLFKKDEESTDEIGLIGIHRFYESGIVFEEYKDYFCISKWYLKEVKRKPYNLKLGCNWQFIPKDEGWTE 440
M81186          SDEFYNTIQIKEYDEQPTYSCQLLFKKDEESTDEIGLIGIHRFYESGIVFEEYKDYFCISKWYLKEVKRKPYNLKLGCNWQFIPKDEGWTE 1291
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